ATRAZINE 080803

Page_____ is not included in this copy.

Pages 253 through 254 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

006937

SEP 21 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Atrazine - Submission of Additional Mutagenicity Data in Response to the Registration Standard. Submitted by Ciba-Geigy. June 19, 1987. Accession No.: 264052: MRID 402466-01.
Tox. Project No.: 7-0902 Tox. Chem. No.: 63

To: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 9/14/87
Section Head, Section VI
and
Irving Mauer, Ph.D. Irving Mauer 9/14/87
Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Review a recently conducted Ames Salmonella assay conducted on atrazine and consider arguments presented by Ciba-Geigy to support the adequacy of two previously reviewed mutagenicity assays (UDS in rat hepatocytes and dominant lethal in mice) that were judged by Toxicology Branch to be unacceptable.

Conclusions/Recommendations:

1. ~~Ames Salmonella Assay: Acceptable and negative up to 5000 ug/plate (the "limit dose" at which slight toxicity was observed).~~ MRID 402466-01

2. UDS Assay in Rat Hepatocytes: Ciba-Geigy's responses are acceptable, as provided by data in the supplement to the original report (dated October 14, 1986). This study is now considered to be acceptable. MRID 402466-02

3. ~~Dominant Lethal Assay in Mice: Ciba-Geigy's responses are not acceptable for concluding that atrazine was adequately tested in this assay. An acceptable assay in the category of chromosomal aberrations is still a data gap for atrazine.~~

~~A detailed review follows.~~

Detailed Review

006937

Study Title: Bacterial Salmonella Mammalian - Microsome Mutagenicity Test

Author: E. DeGrace

Report Dated: December 5, 1984

Conducting Laboratory: Ciba-Geigy Limited, Basle, Switzerland

Study Number: 86[illegible]

Test Material: G 30027 Technical; purity, 98.2%; Batch No., Lot 210200

Vehicle: Dimethylsulfoxide

Procedure: The assay was conducted according to the methods of Ames, et al. (ref. 1-3). The strains tested were TA 98, TA 100, TA 1535 and TA 1537 both with and without metabolic activation. The metabolic activation system was derived from the S9 fraction of liver from Tif:RAIf(SPF) rats treated with Aroclor 1254. Positive controls consisted of the following:

Without metabolic activation

TA 98	daunomycin-HCl
TA 100	4-nitroquinoline-N-oxide
TA 1535	sodium azide
TA 1537	9(5)-aminoacridine hydrochloride monohydrate

With metabolic activation

TA 98	2-aminoanthracene
TA 100	2-aminoanthracene
TA 1535	cyclophosphamide
TA 1537	2-aminoanthracene

A signed quality assurance statement was included with the report. Historical control data for each strain were also included.

Preliminary Toxicity Screen: The toxicity screen was conducted with TA 100 in the absence of metabolic activation. Concentrations ranged from 0.08 to 5000 ug/0.1 ml. One/tenth of an ml was added to each plate.

Concentrations of Test Material: 20, 78, 313, 1250 and 5000 ug/0.1 ml both with and without metabolic activation.

Criteria for a Positive Response: (The following is taken directly from the report).

The test substance is considered to be positive in this test system if one or both of the following conditions are met:

- a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration level for one or more of the following strains: TA 98, TA 1535 and TA 1537,

- a reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by a factor of 1.5 for strain TA 100.

Generally a concentration-related effect should be demonstrable.

Results: In the preliminary toxicity screen, no toxicity was seen up to 5000 ug plate (the limiting dose) and this was, therefore, chosen as the highest dose to be tested.

No increase in the incidence of histidine-prototrophic mutants in comparison with the negative control was seen with any of concentrations of G 30027 tested. Minimal toxicity was seen at 5000 ug/plate for all strains tested both with and without metabolic activation.

Conclusions: This is an acceptable assay, indicating that G 30027 is negative when tested up to 5000 ug/plate in the Salmonella/ mammalian - microsome mutagenicity test.

Study Title: Atrazine: Autoradiographic DNA Repair Test on Rat Hepatocytes

This study was completed on May 16, 1984 and submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and their comments were transmitted to Ciba-Geigy by letter on April 23, 1987. The study was considered to be unacceptable for the following reasons:

a. "The combined 24-hour hepatocyte attachment period and 5-hour test compound exposure time caused a marked reduction in assay sensitivity as indicated by the less than adequate response of the positive control;
b. Cytoplasmic background grain counting was not performed;
c. Slides were not coded".

Ciba-Geigy has submitted arguments/additional data to address the above points. These include:

a. "Both time periods used appear to be appropriate in that the positive control assays provided a clear, consistent positive response. These responses, as well as a number of historical control values can be compared in the supplement to the report [The supplement was included in the submission.];
b. Cytoplasmic background grain counts are provided in the supplement to the report [The supplement was included in the submission.];
c. The slides were not coded; however, they were counted electronically there eliminating bias".

After reviewing the submitted data, Toxicology Branch concludes that the assay

[illegible] considered acceptable for showing that G 30027 does not induce [illegible] in mice.

Study Title: Atrazine: Dominant Lethal Test

This study was originally submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and found to be unacceptable. Our comments were transmitted to Ciba-Geigy by letter on April 23, 1987. With this submission, Ciba-Geigy has responded to our comments indicating that they believe that the assay should be considered acceptable.

Toxicology Branch Comment:

Under the conditions of the study, 444 or 1332 mg/kg G30 027, administered by gavage to male mice, did not elicit a dominant lethal effect. However, G30 027 did not induce a toxic or a cytotoxic effect. Therefore, we are unable to assess whether the test material reached the target organ (gonads). Ciba-Geigy's Response:

There is nothing in the TSCA test guideline requirement (40CFR part 798.5450) citing the need for determining whether the test substance reached the gonads. (A systemically administered compound would be expected to reach all organ systems.)

Toxicology Branch Comment:

Although performed and reported in 1981, this study was not submitted until 1986, at which time the current Test Guidelines (September 27, 1985) had been in effect for at least a year. According to these current Guidelines ("at least'....3 dose levels are employed, the highest should produce signs of toxicity, either clinical (in treated males), or reduced fertility (in untreated pregnant females)." Neither procedure was followed in this test, and no evidence given that any portion of the orally administered single dose reached all organ systems in effective amounts to cause either target toxicity or mutagenic events).

Ciba-Geigy Response:

As stated in a footnote on page 5 of the report, the approximate oral LD_{50} of atrazine in mice is 3992 mg/kg. The present study conducted at levels of 1332 and 444 mg/kg used doses that were 33 and 11 percent of the LD_{50} dose, respectively. The dose of 1332 mg/kg would be expected to elicit systemic toxicity based on findings of sedation, dyspnea, piloerection, and hunched appearance observed in mice treated with a dose of 1871 mg/kg (see refereence 1). From these data, it would appear that the maximum tolerated dose would be exceeded at a level of 1332 mg/kg.

Toxicology Branch Comment:

The high dose of 1332 mg/kg is indeed quite high, but produced no effects in any of the treated males. The selection of doses was stated to have been based on Reference 1 (attached to the back of the report): "Acute

[illegible] Test, Mutation in the "Mouse", an unpublished study conducted with a [illegible] of test chemical with an unstated purity and, hence, not appropriate for dose selection.

Citizenship Response:

In the September 24, 1986 Federal Register (Vol. 51, No. 186, p. 34009), it is stated that "the Agency will place greater weight on tests conducted in germ cells than in somatic cells, on tests performed in vivo rather than in vitro, in eukaryotes rather than prokaryotes, and in mammalian species rather than in submammalian species." The attached test report fulfills or exceeds those criteria.

Toxicology Branch Comments:

The "criteria" described here refer to an overall assessment of mutagenic potential for man, and not to a scientific evaluation of any particular mutagenicity assay (where current Test Guidelines govern).

Further, this dominant lethal study is deficient in not sampling the entire spermatogenic cycle of the mouse, acknowledged by seasoned investigators as more closely approximating 8 weeks, not the six weeks of matings employed in this study. That 8 weeks of mating was standard practice even in the laboratory is re-enforced in the additional data submitted as a supplement (starting on p. 52 of the present version of the study report), on a positive control group performed in the same year as this atrazine study, detailing the results of 8 weeks of mating.

Conclusion:

This study remains unacceptable for the reasons outlined in the Toxicology Branch comments above.

References:

1. Ames, BN, Lee, FD and Durston, WE (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.

2. Ames, BN, Durston, WE, Yamasaki, E and Lee, FD (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. USA 70, 2281-2285.

3. Ames, BN, McCann, J and Yamasaki, E (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test. Mutation Res. 31:347-364.

WC. 5833

EPA: 68-02-4225
DYNAMAC No. 230A-6
February 27, 1987

006937

DATA EVALUATION RECORD

ATRAZINE

Mutagenicity--Unscheduled DNA Repair in Primary Rat Hepatocytes

STUDY IDENTIFICATION: Puri, E. and Muller, D. Autoradiographic DNA repair test on rat hepatocytes with G 30 027, technical. (Unpublished study No. 831171 prepared and submitted by CIBA-GEIGY Ltd., Basle, Switzerland; dated May 16, 1984.) Accession No. 284052. MRID 40246602

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-27-87

006937

1. CHEMICAL: Atrazine; G 30 027.

2. TEST MATERIAL: G 30 027 technical was from batch No. P 210200 and had a purity of 98.2%; no further details were provided.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA repair in primary rat hepatocytes.

4. STUDY IDENTIFICATION: Puri, E. and Muller, D. Autoradiographic DNA repair test on rat hepatocytes with G 30 027, technical. (Unpublished study No. 831171 prepared and submitted by CIBA-GEIGY Ltd., Basle, Switzerland; dated May 16, 1984.) Accession No. 284052.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-27-87

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy for
Date: 2-27-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-27-87

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 3/9/87

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 3/12/87

In support of this recommendation, Barfknecht et al.[5] recently demonstrated that an 18-hour exposure period was superior to shorter intervals for detecting the DNA repair elicited in response to chemicals such as 4-nitroquinoline-1-oxide, mitomycin C, and dimethylnitrosamine, which are well known for their DNA-damaging activity.

It is noteworthy that the concentration of DMN (100 mM) selected by the authors to demonstrate a positive 13.5-fold increase in UDS over the control was approximately 1000 times higher than the level used by Barfknecht et al. ($1x10^{-4}$ M) to affect a response 4 hours posttreatment, comparable to that reported in this study. The latter showed that after 18 hours, $1x10^{-4}$ M DMN induced a 40-fold increase. From a comparison of the study authors' results and those of Barfknecht et al. using DMN, we assess that the combination of a 24-hour attachment period and a 5-hour exposure period caused a marked reduction in assay sensitivity. Of equal concern is the extreme difference in the positive control concentration and the highest assayed dose of G 30 027; the DMN dose was 50 times higher than the highest concentration of the test material (150 µg/mL).

3. Cytoplasmic background grain counts were not counted. These data are essential because silver grains are not homogeneously distributed over the slides; therefore, the only way to correct for random grain distribution is to count and subtract the grains of adjacent cytoplasmic areas from the nuclear counts.

4. The slides were not coded.

We conclude, therefore, that the study should be repeated in accordance with recommended procedures for the primary rat hepatocyte UDS assay.

Item 15--See footnote 2.

16. CBI APPENDIX: ~~Appendix A, Material and Methods,~~ CBI pp. 5-7.

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[5] Barfknecht, T. R., Naismith, R. W. and Kornburst, D. J. Variations on the standard protocol design of the hepatocyte DNA repair assay. (Manuscript submitted to the J. Appl. Toxicol.)

TABLE 1. Representative Results of the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay with G 30 027

Substance	Dose	No. of Cells Scored	Average Silver Grains/ Nucleus
Negative Control Untreated cells	-	150	1.61
Solvent Control Ethanol	-	150	1.61
Positive Control Dimethylnitrosamine	100 µM	150	21.8[a]
Test Material G 30 027	150 µg/mL[b]	150	1.42

[a]Positive by authors' criterion (>2-fold increase in the number of silver grains/nucleus over the control).

[b]Highest dose assayed; compound precipitation was reported for this dose in the preliminary cytotoxicity assay. Values for lower doses (1.2, 6, and 30 µg/mL) were slightly lower than the controls.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The seven doses of the test material examined in the preliminary cytotoxicity assay ranged from 3.125 to 150 µg/mL. No cytotoxicity was reported for the selected concentrations; however, the report stated that at the highest dose assayed, slight compound precipitation was observed.

B. UDS Assay: The UDS assay was conducted with 1.2, 6, 30, and 150 µg/mL of the test material. The choice of this dose range was based on the results of the preliminary cytotoxicity assay.

The authors did not report compound precipitation at the high dose. No evidence of a cytotoxic effect was observed at any dose, and the mean silver grains/nucleus for all doses were either comparable to or slightly lower than the control values. Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated, "It is concluded that, under the given experimental conditions, no evidence of induction of DNA damage by G 30 027 or by its metabolites was obtained that could be interpreted as suggestive of mutagenic or carcinogenic properties of the substance."

B. A quality assurance statement was signed and dated May 10, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study is unacceptable for the following reasons:

1. Since the attachment period was prolonged (i.e., 24 hours), it is likely that the metabolic activity of the cells was reduced; hence, cells with less than adequate sensitivity were exposed to the test material. The recommended attachment period is 1.5 to 2 hours.[3]

2. The length of exposure of the hepatocytes to the test material (5 hours) may have been too short to induce a UDS response. The U.S. Environmental Protection Agency Gene-Tox Program[4] recommends an 18-hour exposure.

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[3] Mitchell, et al. (1983). Mutat. Res. 123(1983):363-410.

[4] Ibid.

Freshly isolated cells were suspended in Williams' Medium E (WME), counted, and dispensed (10^5 cells/mL) onto gelatinized coverslips in multi-well culture plates. The cultures were allowed to attach to the coverslips for 1.5-2.0 hours under unspecified conditions. Prior to use in the assay, attached cells were washed, refed fresh medium, and incubated overnight.

3. Preliminary Cytotoxicity Assay:

Cells were exposed to seven concentrations of the test material and the solvent control for 5 hours. Dosed cells were rinsed, stained with Trypan blue, and fixed, and the percentage of unstained cells in 100 scored hepatocytes was determined. The following criteria were used to evaluate the cytotoxicity results and to establish doses for the UDS assay: a sufficiently large number of cells must adhere to the coverslip, at least 25% of the cells must show viability upon examination by means of the vital-staining techniques, and a corresponding percentage of the cells must display normal morphological characteristics.

5. UDS Assay:

a. Treatment: Four preselected concentrations of the test material were evaluated in the UDS assay. Quadruplicate cultures per group were exposed to the test material doses, the negative control (untreated), the solvent control (ETOH), and 100 mM dimethylnitrosamine (DMN), the positive control, in the presence of 1 µCi/mL [^{3}H]-thymidine for 5 hours. Exposed cells were washed and fixed with ETOH/acetic acid (3:1) and the coverslips were mounted onto slides.

b. Preparation of Autoradiographs/Grain Development: The procedures and solutions used to develop the nuclear grains were not described; the report stated, however, that the exposure time was 6 hours. Autoradiographs were stained with hematoxylin-eosin. The report did not indicate whether the slides were coded.

c. Grain Counting: Nuclear grains of 150 cells for each of the dose and control groups were counted. The background count was determined in cell-free areas and the reported stated, "It was found to be negligibly low."

6. Evaluation Criteria: The assay was considered positive if the mean nuclear grain count was >2-fold higher than the negative control at any dose.

B. Protocol: A protocol was not presented.

7. CONCLUSIONS:

A. The primary rat hepatocyte unscheduled DNA synthesis (UDS) assay conducted with G 30 027 technical is unacceptable for the following reasons:

1. The combined 24-hour hepatocyte attachment period and 5-hour test compound exposure time caused a marked reduction in assay sensitivity as indicated by the less than adequate response of the positive control (see Reviewers' Discussion).

2. Cytoplasmic background grain counting was not performed.

3. Slides were not coded.

B. The study is unacceptable.

8. RECOMMENDATIONS:

The repeat assay should be performed in accordance with recommended procedures.[2]

Items 9 and 10—See footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: G 30 027 was from batch No. P 210200 and had a purity of 98.2%. No information on the physical appearance, stability, or storage conditions was provided. The test material was dissolved in ethanol (ETOH).

2. Indicator Cells: Primary rat hepatocytes were collected from a male rat (Tif:RAIf, SPF); the method used to harvest the hepatocytes was not reported. The rat was obtained from CIBA-GEIGY Tierfarm, Sisseln.

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[1]Mitchell, A. D., Casciano, D. A., Meltz, M. L., Robinson, D. E., San, R. H. C., William, G. M., and Von Halle, E. S. Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983):363-410.

[2]Only items appropriate to this DER have been included.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-04

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87048

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Mnelo Park, CA 94025 (Study No. LSC-1469)

TITLE OF REPORT: Disposition of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine

tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: Not provided in this summary report.
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be ≥ 98% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 160-225g
Source: Charles River Breeding Laboratories, Portage, MI (refer to p. 59 of this study).

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Elimination and Distribution Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats male	Rats female	Duration of Exposure
1 Control	0.0	2	2	none
2 Low	1.0	5	5	1 day
3 High	100.0	5	5	1 day
4 SubQ	1.0	5	5	15 days

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days.

Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from SRI International, the subcontracting laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, the analytical phase of the metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because: (1) "there was no QA [quality assurance] inspection of the analytical phase of the study" and (2) " there was no QA audit of [this] final report ABR-87042."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the distribution (and elimination) of atrazine.

As shown in Table 1, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine. A control group received vehicle only.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were measured in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

Digestive system		**Cardiovascular**		**Neurological**			
	Tongue		Aorta*	X	Brain*+@		
	Salivary glands*	X	Heart*@		Peripheral nerve*#		
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#		
	Stomach*		Lymph nodes*	X	Pituitary*		
	Duodenum*	X	Spleen@		Eyes (optic n.)*#		
	Jejunum*		Thymus*				
	Ileum*	X	Red blood cell		**Glandular**		
	Cecum*	**Urogenital**			Adrenal gland*		
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#		
	Rectum*		Bladder*	X	Mammary gland*#		
X	Liver *+@	X	Testes*+@		Parathyroids*++		
	Gall bladder*#		Epididymides		Thyroids*++		
	Pancreas*		Prostate	**Other tissues**			
Respiratory			Seminal vesicle	X	Bone (femur)*#		
	Trachea*#	X	Ovaries*+@	X	Muscle*#@		
X	Lung*@	X	Uterus*@		Skin*#		
	Nose^		Cervix		All gross lesions and masses*		
	Pharynx^		Fallopian tubes	X	Residual Carcass@		
	Larynx^			X	Fat@		
				X	Plasma (blood)@		

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution and Elimination of Atrazine and Its Metabolites

Five male and 5 female rats were used to assess the disposition and elimination of atrazine after acute or subchronic exposure. Table 2 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the subchronic group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 2). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats subchronically treated with atrazine when compared to the group of rats who received a single dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding ^{14}C-label in the urine, feces, and the tissues measured 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 3). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the subchronic group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the low dose group. The ratios, 1.11 and 1.00, also provide some indication that atrazine and its metabolites had not accumulated in the red blood cells or any other tissues

under this exposure regimen. This assertion is based on the observation that tissue concentrations were the same in the acute and subchronic exposure groups (Table 3).

The high concentration of ^{14}C-label reported in the red blood cell is discussed in further detail. The author suggests that ^{14}C-label binding in the red blood cell is the product of a covalent interaction between the triazine moiety of ^{14}C-atrazine and the cysteinal sulfhydryl groups in the rat hemoglobin macromolecule.

The remaining tissues listed in Table 3 show lower levels of ^{14}C-atrazine and its metabolites. Also, in these tissues, the ratio of ^{14}C-label binding was proportional lower than the administered dose, e.g., ^{14}C-label concentrations in the subchronic group were lower than that for the acute exposure group (Table 5). This finding provides evidence that atrazine or its metabolites appear not to accumulate in any tissues under this exposure regimen. However, cumulative binding of atrazine metabolites in RBCs after chronic exposure may occur.

Toxicokinetic modeling. The whole body half-life ($t_{1/2}$) of ^{14}C-label was 31.3 ± 2.8 hours (1.3 days) was calculated from the urinary excretion data. The author reported that the data best fits an open two-compartment toxicokinetic model. In addition, no statistically significant differences were reported between treatment groups or sex regarding the values for: alpha, beta, k_{10}, k_{12} and k_{21} or the whole body $t_{1/2}$ value.

Table 2
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment (taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash[a]	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

[a] At sacrifice, the cages were washed with a water acetone mixture (1:1) and 10 ml aliquots were measured for radioactivity.

Table 3
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment
(taken from Table IV)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
RBC	0.559	0.627	67.536	62.366	0.662	0.628
Kidney	0.229	0.263	6.936	6.990	0.155	0.140
Liver	0.247	0.498	7.378	7.468	0.204	0.212
Brain	0.166	0.162	5.210	4.580	0.076	0.070
Gonads	0.147	0.198	5.124	5.799	0.066	0.050
Heart	0.144	0.154	11.726	9.770	0.137	0.102
Spleen	0.136	0.148	10.748	12.563	0.156	0.169
Lung	0.115	0.134	9.229	9.128	0.111	0.132
Pituitary	0.080	0.081	4.126	4.220	0.088	0.074
Carcass	0.076	0.080	6.349	5.901	0.069	0.061
Muscle	0.060	0.067	4.080	3.637	0.044	0.041
Bone	0.044	0.047	3.476	3.625	0.042	0.038
Fat	0.015	0.011	1.245	1.320	0.014	0.009
Plasma	0.009	0.010	1.200	1.039	0.011	0.013
Mammaries	-	0.005	-	0.346	-	0.006
Uterus	-	0.033	-	3.743	-	0.047

V. **DISCUSSION:**

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

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2Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-05

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87087

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 (Study No. LSC-1469)
-and-
Agrisearch Incorporated, 26 Water Street, Frederick, MD 21701 (Project No. 1271)

TITLE OF REPORT: Study of delta-^{14}C-Atrazine Dose/Response Relationship in the Rat (General Metabolism).

AUTHOR: B. Thede

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red

blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: S85-0653-J
Purity: 98.8% (expiration date - November, 1990)
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound (reference CL-IX-77) was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be 97.9% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 243.2g ± 2.7 SE (240-265g)
Source: Charles River Breeding Laboratories, Wilmington, MA

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Mid1 (MDT1)	3.0	2	10
4 Mid2 (MDT2)	7.0	2	10
5 Low3 (MDT3)	10.0	2	10
6 Mid4 (MDT4)	50.0	2	10
7 High (HDT1)	100.0	2	10

a After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days. Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

Table 4
Tissue Levels of ^{14}C-Label (ppm) at Sacrifice
(taken from Table X)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Tissue:												
Liver	2.97	2.33	5.40	8.06	16.21	8.05	20.86	12.37	54.87	32.58	102.37	55.8
Pituitary	1.18	0.50	1.67	3.54	7.32	3.24	9.36	4.49	36.91	18.18	71.68	33.9
Ovary	1.14	0.48	1.59	3.62	7.28	2.81	9.08	4.69	36.30	17.42	76.39	33.0
Brain	0.39	0.24	0.90	1.57	3.24	1.55	4.12	2.04	14.59	8.99	30.25	11.8
Kidney	1.36	0.61	1.64	3.54	6.91	3.35	9.88	4.70	29.31	16.73	78.64	26.1
Mammaries:												
Pectoral	0.13	0.05	0.38	0.46	0.52	0.24	1.24	0.75	4.41	3.32	6.30	7.3
Inguinal	0.06	0.05	0.15	0.19	0.79	0.11	0.54	0.65	4.06	2.29	4.77	0.3

V. DISCUSSION

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404313-06 and the data evaluation report on MRID No. 404313-06, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated obstensibly in MRID No. 404313-06. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. Therefore, the major urinary metabolites of atrazine in female rats reported in MRID No. 404313-06 are:

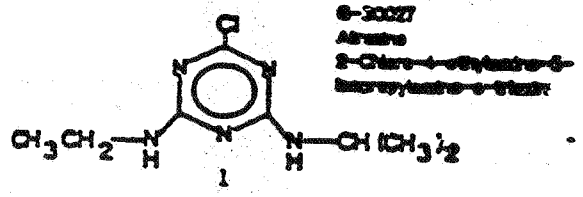
- 2-chloro-4-amino-6-isopropylamino-s-triazine (13),
- 2-chloro-4-ethylamino-6-amino-s-triazine (14), and
- 2-chloro-4,6-diamino-s-triazine (15).

The molecular structures of the above atrazine metabolites are shown in Figure 1 (the numbers in Figure 1 correspond to those associated with the above metabolites). Of the metabolites listed above, 2-chloro-4,6-diamino-s-triazine (15) is reported to be the major urinary metabolite. The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

PC1/reports/atramet1.006

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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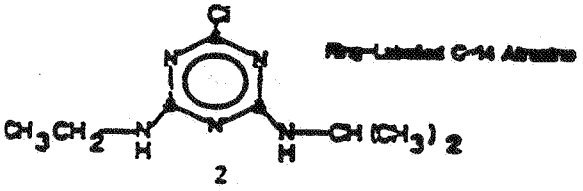

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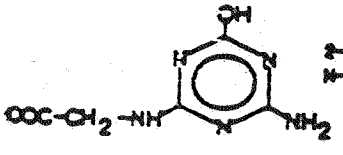

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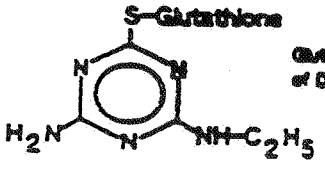

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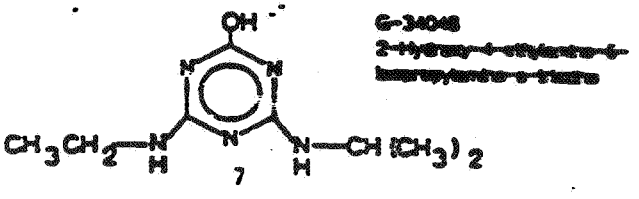

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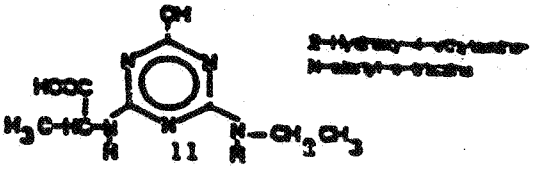

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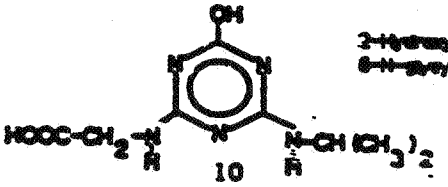

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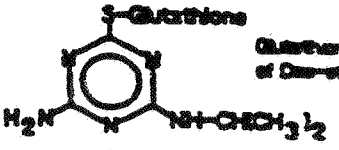

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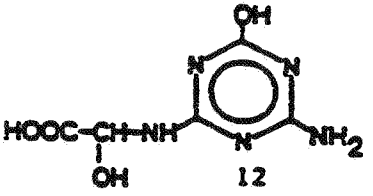

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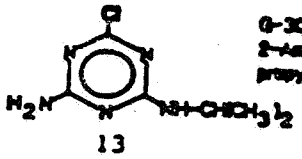

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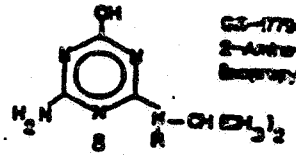

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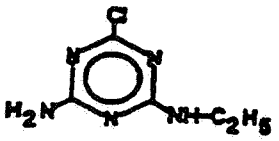

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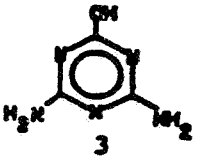

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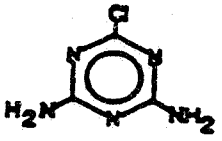

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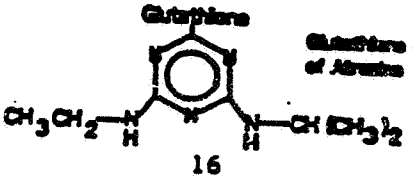

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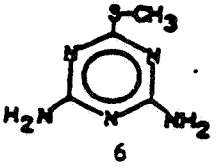

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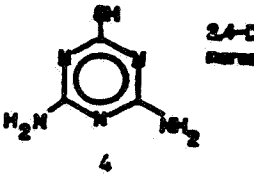

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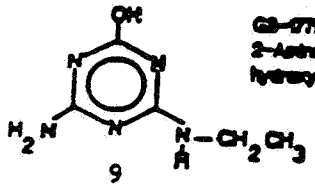

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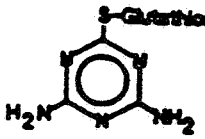

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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

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Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 1.0 microCurie/mg. The purity of the radiolabeled test compound was reported to be ≥ 97%.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley
Age: Not provided in this report.
Weight (mean): about 0.2 kg
Source: Harlan Sprague-Dawley, Indianapolis, IN

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 24 hours. Animals were individually placed in metabolism cages for the collection of urine.

B. Dose Method: The rats were allowed a 5-day acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 1% methyl carboxymethyl cellulose and Hi-Sil-233 brand of powdered silica used to suspend the atrazine in solution. The rats were allowed free access to animal feed (Purina) and deionized water.

C. Statistics:

No statistical procedures were used in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, this metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because:

(1) "A complete set of biological phase SOPs have not been established.

(2) There was no QA inspection of the study because the QAU was not a fully functional unit at the time the study was conducted.

(3) There was no QA audit of the final report ABR-87115."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to identify the atrazine metabolites in two groups of rats.

As shown in Table 1, one group of female rats was given a single dose of atrazine in an effort to produce sufficient levels of urinary metabolites of atrazine for identification. Five adult female Sprague-Dawley rats (about 0.2 kg) were administered 100 mg/kg ^{14}C-atrazine. Samples of urine and feces were obtained at 24, 48, and 72 hours. After taking samples for 72 hours, the rats were sacrificed and 5 ml of blood and the liver were obtained.

In another group of animals, 8 rats were given a single oral exposure of 16.18 - 19.64 mg/kg ^{14}C-atrazine. Urinary metabolites were collected over a 24-hour period following treatment. The metabolites of atrazine were isolated and identified by the following series of analytical chemistry steps:

(1) charcoal cleanup,

(2) C_{18} Bond-Elut separation,

(3) Aminex A-4 cation exchange column chromatography,

(4) Aminex A-25 anion exchange column chromatography or PRP-1 (reverse-phase) HPLC, and finally

(5) confirmation by comparing to the infrared spectra and mass spectra of authentic synthesized standards.

V. RESULTS:

A. The In Vivo Metabolism of Atrazine.

To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label. The amount of ^{14}C-label eliminated via exhalation was not reported.

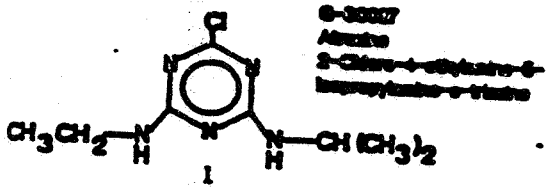
The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Metabolites were then dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Only four of these metabolites were identified and were reported, they were:

- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the four metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because several other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12),

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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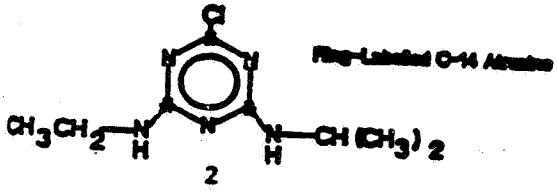

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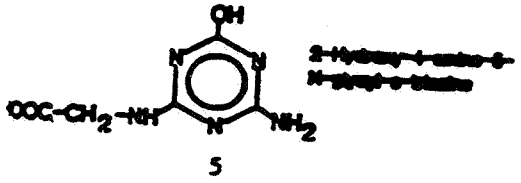

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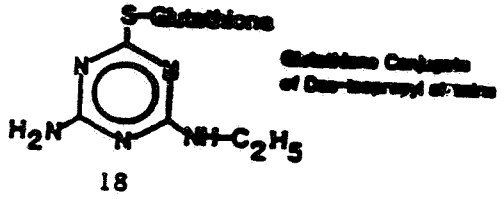

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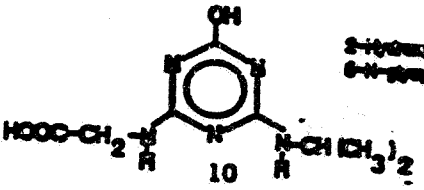

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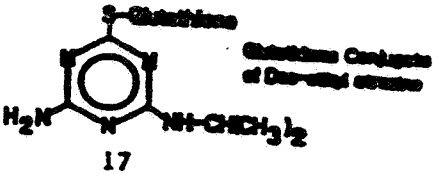

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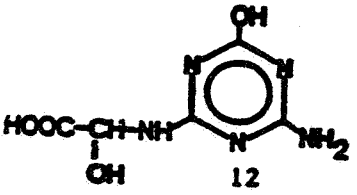

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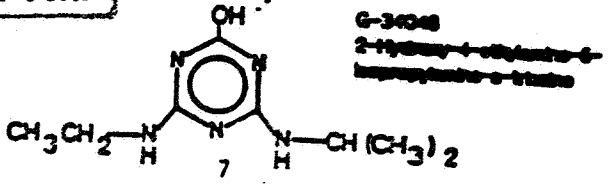

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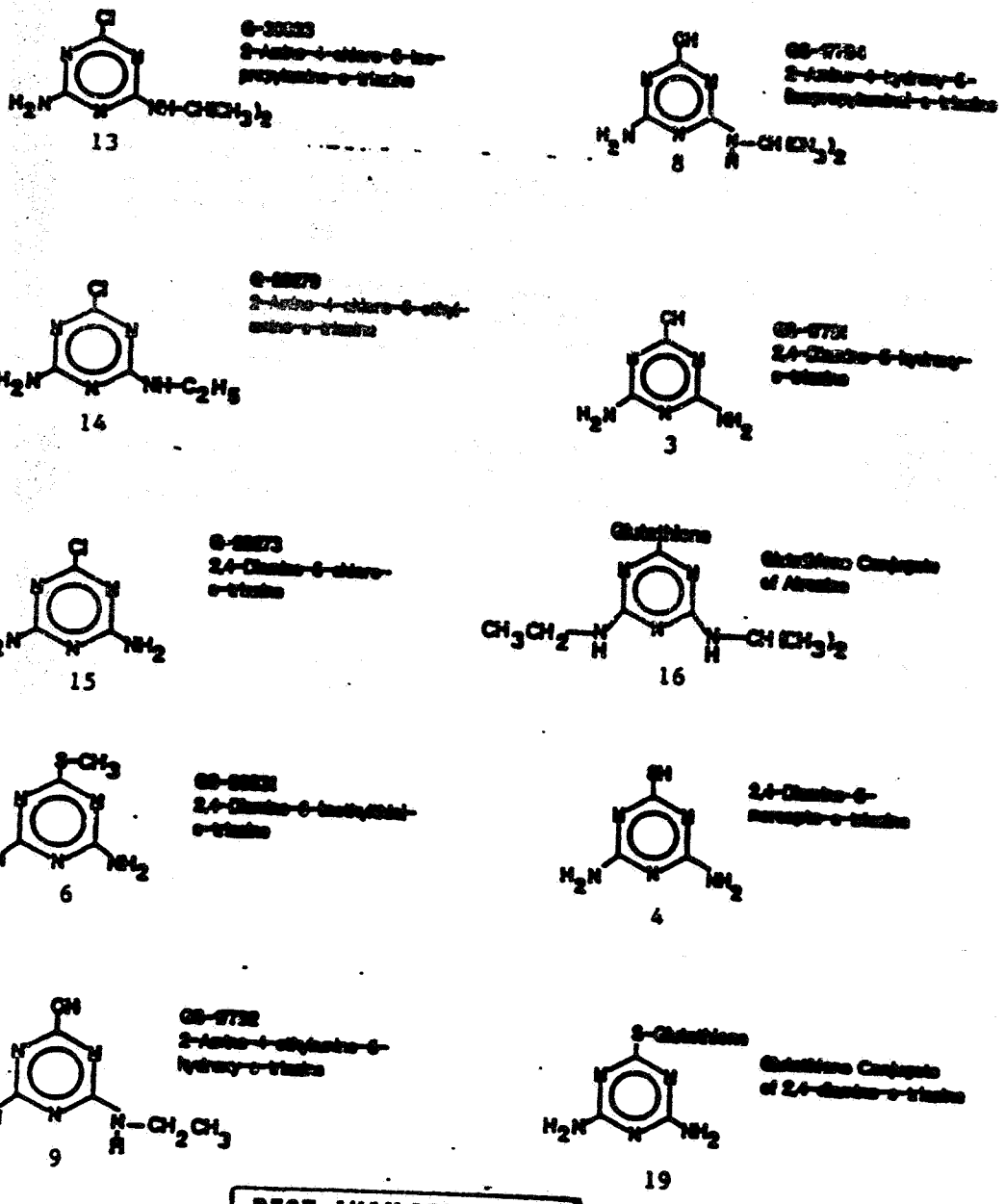

FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.

B. The In Vitro Metabolism of Atrazine.

The author of this study offers the results of a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding of atrazine in RBCs.

The method published by Dauterman and Muecke is reported as the following steps. Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards. The results corroborate the findings in the in vivo experiment that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites previously discussed when cytosolic cell fractions were included in the in vitro reactions.

Covalent binding in RBCs. The author argues that the glutathione-containing metabolites of atrazine may be catalyzed by a "carbon-sulfur lyase," an enzyme that cleaves the glutathione residue and leaves a thiol group on the atrazine metabolite. However, the author has not presented evidence whether lyase is present in red blood cells.

V. **DISCUSSION:**

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

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Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-09

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-85104

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Metabolism of ^{14}C-Atrazine in Orally Dosed Rats (General Metabolism).

AUTHOR: B.J. Simoneaux

REPORT ISSUED: December 6, 1985

CONCLUSIONS:

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound:

Description: Atrazine
Batch #: Not reported in this study
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound were 13.5 microCuries/mg and 12.9 microCuries/mg for the low and high dose groups, respectively. The purity of the radiolabeled test compound was reported to be $\geq$ 97.5% ascertained by a thin-layer chromatography system.

B. Test Animals:

Species: Rat (male)
Strain: Harlan Sprague-Dawley
Age: Not provided in this report.
Weight (mean): 250g
Source: Harlan Madison, WI

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats (male)	Day of Sacrifice	Duration of Exposure (days)
2 Low	0.4	3	5	4
Low	0.4	3	7	7
Low	0.4	3	9	7
Low	0.4	3	10	7
Low	0.4	3	14	7
Low	0.4	3	18	7
3 High	4.0	3	5	4
High	4.0	3	7	7
High	4.0	3	9	7
High	4.0	3	10	7
High	4.0	3	14	7
High	4.0	3	18	7

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured at 24-hour intervals in the group of rats exposed for 18 days. Animals were individually placed in metabolism cages for the collection of feces and urine. There was no control group.

B. Dose Method: Atrazine was given orally (via a stomach tube) to the rats as a radiolabeled active ingredient. The 250 g rats were given 0.1 mg/rat (low dose) or 1.0 mg/rat (high dose). The vehicle was in the aqueous Carbowax-200 (PEG 200) formulation (0.3 ml ethanol:0.2 ml water:0.5 ml PEG 200). The rats were allowed free access to animal feed and tap water.

C. Statistics:

The following procedure was utilized in analyzing the numerical data:

The SOP method of Wolf and Sumner, AG-276, "Statistical methods in the measurement of radioactivity" were used to calculate ppm-equivalents of the ^{14}C-label obtained from the rats.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the metabolism of atrazine.

Three male rats in each group were repeatedly dosed and then sacrificed 5, 7, 9, 10, 14, and 18 days after dosing was initiated (for details, see Table 1). Urine and feces were collected for analysis from the rats exposed for the 18-day period. The rats were sacrificed and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

Digestive system		**Cardiovascular**		**Neurological**	
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
X	Stomach*		Lymph nodes*		Pituitary*
X	Duodenum*		Spleen@		Eyes (optic n.)*#
X	Jejunum*		Thymus*		
X	Ileum*	X	Red blood cell	**Glandular**	
	Cecum*	**Urogenital**			Adrenal gland*
X	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*		Mammary gland*#
X	Liver *+@		Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate	**Other tissues**	
Respiratory			Seminal vesicle		Bone (femur)*#
	Trachea*#		Ovaries*+@	X	Muscle*#@
	Lung*@		Uterus*@		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes	X	Residual Carcass@
	Larynx^			X	Fat@
				X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

[FIFRA Subdivision F test guidelines §85-1 (e)(3)(i) require that, in addition to the tissues listed in Figure 1 above, the levels of atrazine or its metabolites shall be measured in the testes, heart, lung, spleen and uterus.]

V. RESULTS:

B. The Metabolism of Atrazine

To examine the metabolism of atrazine in rats, two doses were employed, 0.4 and 4.0 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-label was measured in selected tissues and in the rats exposed for 18-days, urinary and fecal levels of ^{14}C-label were monitored. A recovery of 103.9% and 93.4% was found for the low and high dose groups, respectively. For the low and high dose groups, respectively, the urinary route accounted for 72.74% and 67.2% of the elimination whereas 27.79% and 23.92% of the ^{14}C-label was eliminated via the fecal route. The author reports that about 95% of the administered dose is eliminated within 48 hours after the last exposure.

The tissues contained the remainder of the ^{14}C-label (Tables 2 and 3). The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC (1.95 ppm) followed by liver (1.10 ppm), kidney (0.74 ppm) and brain (0.38 ppm) and are listed in Table 2. The highest tissue levels of ^{14}C-label, in decreasing order, in the high dose group of rats at 8 days were found as such: RBC (21.66 ppm), liver (6.40 ppm), kidney (5.28 ppm) and brain (2.48 ppm). In general, 10 days after the last dose of ^{14}C-atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of ^{14}C-label remaining. The remaining tissues had lower levels of ^{14}C-label at 8 or 10 days and lower levels remaining at 18 days. The peak tissue levels in the low dose group occurred at 10 day whereas the peak levels in the high dose group was reported at 8 days.

As percentage of administered dose (Table 3), the muscle had the highest levels followed by the liver and RBC. Percentage of tissue levels were highest in those rats sacrificed 4 days after initial atrazine exposure (Table 3).

Table 2
Tissue Levels of ^{14}C-Label (ppm) Remaining After Sacrifice
(taken from Table IV)

	Time of Sacrifice (Days)					
0.1 mg/rat	4	6	8	10	14	18
Plasma	0.06	0.05	0.06	0.04	0.01	0.01
RBC	1.11	1.18	1.63	1.95	1.31	1.53
Fat	0.04	0.04	0.04	0.05	0.05	0.03
Brain	0.29	0.30	0.39	0.38	0.27	0.24
Muscle	0.13	0.13	0.15	0.15	0.12	0.11
Kidney	0.67	0.63	0.74	0.71	0.32	0.23
Liver	0.88	0.91	1.06	1.10	0.56	0.40
Stomach	0.20	0.66	0.21	0.18	0.10	0.10
Small Intestine	0.21	0.24	0.26	0.15	0.06	0.09
Large Intestine	6.17	0.25	0.20	0.16	0.07	0.09
Large Intestinal Content	0.80	0.87	0.64	0.30	0.11	0.12
1.0 mg/rat						
Plasma	0.55	0.82	1.02	0.37	0.14	0.07
RBC	9.30	16.34	21.66	17.17	15.50	13.78
Fat	0.21	0.28	0.43	0.23	0.15	0.19
Brain	1.23	2.10	2.48	1.76	1.39	1.14
Muscle	0.77	1.27	1.56	0.91	0.83	0.75
Kidney	3.09	4.49	5.28	3.41	1.92	1.26
Liver	4.26	5.48	6.40	4.48	2.87	1.80
Stomach	1.46	2.01	1.96	0.52	0.32	0.15
Small Intestine	1.44	1.87	2.22	0.75	0.32	0.22
Large Intestine	1.28	1.56	1.86	0.97	0.60	0.56
Large Intestinal Content	9.15	12.27	11.94	1.28	7.97	0.22

Table 3
Percent of Dose of ^{14}C-Label Remaining After Sacrifice
(taken from Table III)

	Time of Sacrifice (Days)					
0.1 mg/rat	4	6	8	10	14	18
Plasma	0.15	0.08	0.10	0.05	0.02	0.01
RBC	1.60	1.28	1.39	1.38	1.08	1.11
Fat	0.26	0.20	0.09	0.37	0.16	0.09
Brain	0.12	0.08	0.09	0.08	0.06	0.05
Muscle	3.66	2.65	2.51	2.05	1.81	1.55
Kidney	0.34	0.26	0.23	0.17	0.10	0.06
Liver	2.55	1.94	1.70	1.28	0.85	0.43
Stomach	0.15	0.35	0.10	0.05	0.03	0.02
Small Intestine	0.15	0.18	0.22	0.07	0.03	0.02
Large Intestine	0.04	0.06	0.03	0.02	0.01	0.01
Large Intestinal Content	0.25	0.29	0.13	0.07	0.02	0.02
1.0 mg/rat						
Plasma	0.15	0.13	0.12	0.05	0.02	0.01
RBC	1.48	1.51	1.46	1.38	1.08	0.94
Fat	0.16	0.12	0.13	0.08	0.04	0.06
Brain	0.05	0.06	0.04	0.04	0.03	0.02
Muscle	2.42	2.23	2.04	1.41	1.12	1.00
Kidney	0.18	0.13	0.11	0.09	0.05	0.03
Liver	1.27	0.87	0.72	0.72	0.42	0.25
Stomach	0.08	0.06	0.10	0.03	0.02	0.01
Small Intestine	0.19	0.10	0.09	0.05	0.02	0.01
Large Intestine	0.04	0.02	0.02	0.02	0.01	0.01
Large Intestinal Content	0.38	0.36	0.13	0.04	0.18	0.01

VI. DISCUSSION

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

006937

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

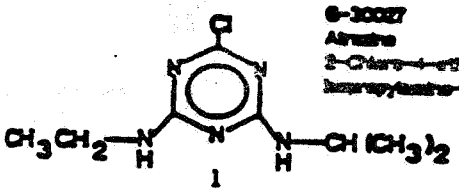
REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404375-01 and the data evaluation report on MRID No. 404375-01, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated superficially in MRID No. 404375-01. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. The major urinary metabolite of atrazine in female rats reported in MRID No. 404375-01 is 2-chloro-4,6-diamino-s-triazine (15). The molecular structure of this atrazine metabolite is shown in Figure 1 (the number in Figure 1 correspond to number '15' with the above metabolites). The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to satisfy completely the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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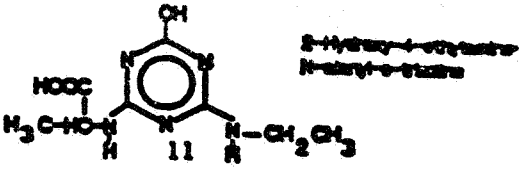

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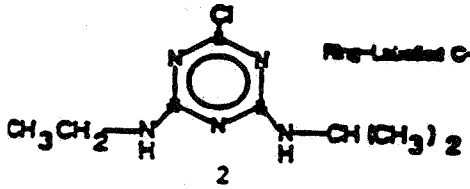

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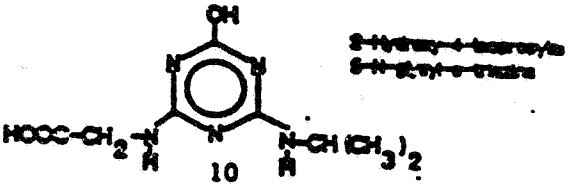

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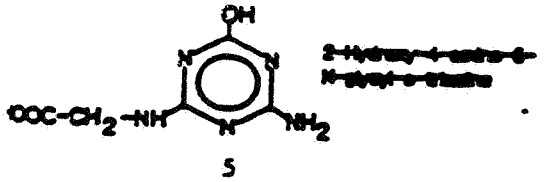

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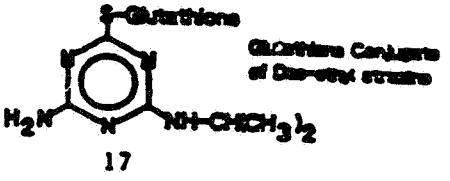

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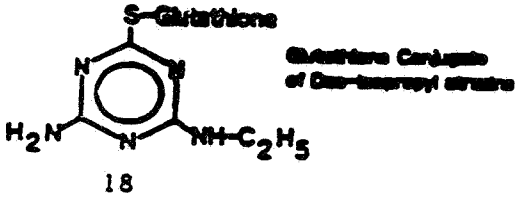

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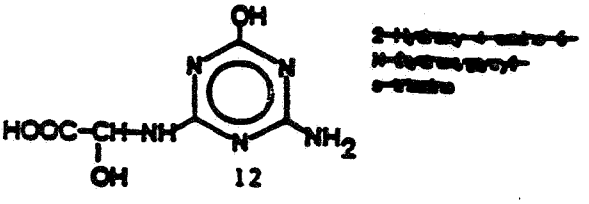

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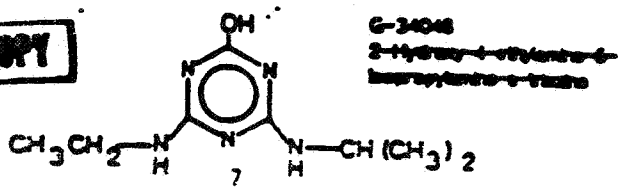

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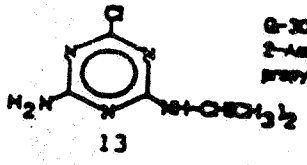

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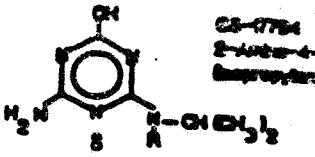

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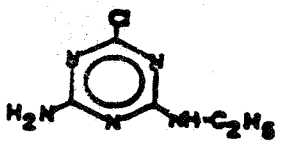

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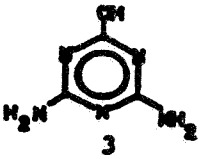

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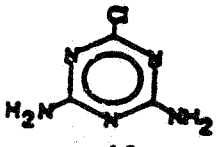

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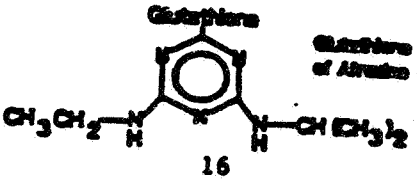

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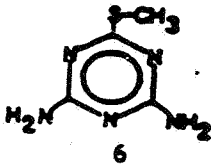

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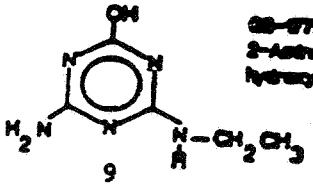

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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006937

DEC 8 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Atrazine Registration Standard: Mutagenicity Testing Requirement

FROM: Kerry L. Dearfield, Ph.D. 12-7-88
Geneticist
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

TO: Marion Copley, D.V.M.
Acting Section Chief
Section 2
Toxicology Branch I - IRS
Health Effects Division (TS-769C)

THRU: John Quest, Ph.D. 12-7-88
Chief
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

12/8/88

Atrazine CAS No. 1912-24-9 Tox. Chem. No. 63

I. Background

As of my previous memo to you dated Aug 19, 1988, Ciba-Geigy had not fulfilled the minimum requirements for mutagenicity testing as required by the OPP. The only acceptable tests that had been submitted fulfilled only one of the three mutagenicity testing categories, i.e. gene mutations (e.g. acceptable Salmonella assay). The two other categories had not been fulfilled, i.e. structural chromosome aberrations and other genotoxic effects. In a subsequent submission from Ciba-Geigy, an acceptable mouse micronucleus test (MRID # 407223-01) was reported and minimally fulfilled the structural chromosome aberration category. A recent Ciba-Geigy letter (dated June 29, 1988) mentioned that a UDS assay in rat hepatocytes they performed was earlier considered acceptable; however, upon rereview, it was downgraded to unacceptable for reasons outlined in a memo dated April 26, 1988 from this reviewer to Robert Taylor. Therefore, one mutagenicity category (other genotoxic effects) remains unfulfilled for registration purposes.

The submitted negative mouse micronucleus assay would now minimally fulfill the structural chromosome aberration category. However, it may still not alleviate our concern about atrazine's potential genotoxicity in vivo. A published report (Adler, Mutat. Res. 74: 77-93, 1980) suggested that atrazine induced a positive increase in aberrations at a dose of 2000 mg/kg by oral gavage in olive oil. The submitted micronucleus test, although found negative, tested to a similar level of 2250 mg/kg by oral gavage in CMC. In another Ciba-Geigy submission in which Dr. David Brusick performs an assessment on atrazine (dated December, 1987), Brusick makes several points relevant here: the micronucleus assay would not totally offset the reported positive bone marrow metaphase assay because (a) the metaphase assay is generally considered to be more sensitive, and (b) the negative assays were performed at roughly equivalent dose levels. Also, vehicle effects may influence the results. The positive study used olive oil and the negative study used CMC. Brusick again points out that a closer inspection of olive oil studies would be valuable and that the bioavailability of atrazine from CMC might be investigated. Overall, it would have been useful to repeat the published study to address the possible concern for atrazine mutagenicity. Additional testing for dominant lethal effects, effects by plant metabolites and possibly aneuploidy was also recommended in the April 26, 1988 memo.

II. Recent communication from Adler on published paper

This reviewer has been in contact with the author of the Adler paper published in Mutation Research. Dr. Adler has sent to OPP the summarized Progress Reports of the atrazine work performed by her and her colleagues. These include summary tables of their data for our evaluation. Summarized progress reports of four studies submitted by Dr. Adler included 1) induction of dominant lethals in male mice, 2) spot test for somatic mutations in mice, 3) chromatid aberrations in mouse bone marrow, and 4) micronuclei in polychromatic erythrocytes of mouse bone marrow. The first three studies are reported positive by the investigators and the micronucleus test negative. It should be noted that raw data and complete protocols were not provided and that Dr. Adler plans to publish this information in 1989. While each of these studies individually would not be classified as acceptable to satisfy the different categories for mutagenicity testing (e.g. incomplete protocols; no report of positive controls; use of only one sex in the micronucleus assay), the data provide enough information to elicit a concern for a possible mutagenicity concern for atrazine. It is the responsibility of the Agency to be aware of such concerns and address them. That was the intention of the April 26, 1988 memo from this reviewer to Robert Taylor when it stated that the reported positive published studies should be examined in more detail. The registrant should address these concerns with acceptable submitted studies.

Atrazine was examined for dominant lethal effects in hybrid male (101xC3H)F_1 mice (investigators were U.H. Ehling and J. Kratochvilova). Male mice were exposed to atrazine at a dose of 2000 mg/kg by oral intubation in olive oil. This dose was lethal to 14% of the exposed mice. Males were mated to females for up to 48 days post-treatment in 4 day mating intervals (new females every 4 days). There was a low frequency of fertile matings in the first mating period (38.6% compared to 96% frequency for the control). However, once pregnant, females appeared to have a comparable number of corpora lutea and implants as the controls. There was a slight increase in dominant lethal mutations in the first 3 mating periods (i.e. first 12 days mating post-treatment) as evidenced by an increase in the percent dead implants over controls.

A spot test was performed to examine for presumed somatic mutations in mice (investigator was A. Neuhauser-Klaus). Embryos were treated in utero with atrazine on the ninth day after conception by oral administration to the mothers. Three experiments were reported. The original experiment administered atrazine in olive oil at single doses of 600, 800, 1000 and 1250 mg/kg. Offspring were examined for color spots at 2-3 weeks after birth and then again at 3-4 weeks. Toxicity was seen as an increase in sterile females up to 800 mg/kg. Higher doses killed some females (precise numbers not provided). However, litter sizes at weaning were not influenced. No evidence in an increase of color spots was evident after atrazine administration up to 800 mg/kg in olive oil (no data at higher dosing). Atrazine was orally administered in corn oil with 0.2% tocopherolacetate at 600 mg/kg in the remaining two experiments. Other conditions appeared similar to the original experiment. In one of these experiments, atrazine increased the number of color spots over control in a statistically significant manner ($p=0.031$), whereas in the other experiment, there was an increase, but was not statistically significant ($p=0.061$). The investigator examined the results in all three experiments and found they were homogenous within control and within experimental groups. Therefore, the data were pooled and a statistically significant difference between controls and treated groups was found ($p=0.007$). The author concluded that 600 mg/kg atrazine induced spots when suspended in oil. Only spots of genetic relevance were analyzed (i.e. white mid-ventral spots not included). While these data do not impact on heritable risk, they suggest there may be a concern for somatic mutations and perhaps for reproductive/developmental effects.

Two in vivo cytogenetic studies were performed with atrazine (investigators were U. Kliesch and I.-D. Adler). Abarrations and micronuclei were assayed in bone marrow from hybrid (101xC3H)F_1 male mice after oral administration of atrazine. The aberration study is the one mentioned above (Part I). A single oral dose of

atrazine at 2000 mg/kg in olive oil was given to 8 animals. Bone marrow was obtained 24 hours after treatment and 125 mitoses/animal scored. An average aberration frequency increase of 4.1% vs. 0.7% for controls was found. Only deletions of the chromatid type were reported. In the micronucleus experiment, a single oral dose of atrazine at 100, 500 or 1000 mg/kg in DMSO was given to 4 animals/dose/sacrifice time. Bone marrow was obtained at 24, 48, 72, 96 and 120 hours after treatment and 2000 polychromatic erythrocytes/animal were scored. Negative results were obtained. In this same report, an additional data entry appears to show that atrazine was also tested in the micronucleus test at a single dose of 2000 mg/kg in olive oil and bone marrow obtained 24 hours after treatment. These results were also negative. The negative micronucleus results appear consistent with the registrant's own micronucleus test results performed in a different mouse strain (MRID #407223-01).

III. Requirements for Registration Standard

As far as minimal requirements for mutagenicity testing is concerned, the category for other genotoxic effects has not been fulfilled. This is a data gap that should be addressed in the Registration Standard. Tests that may be appropriate here may examine for aneuploidy and/or the impact of plant metabolism. Since these are not routine tests, the selection of what test(s) to perform for this category should be discussed with the OPP.

The results from the dominant lethal assay suggest that there may be a concern for heritable risk from atrazine exposure. The reduced fertility frequency indicates that there is exposure to the germ cells in the exposed males. The slight increase in dominant lethal effects suggests that there may be genetic alterations that could be transmissible. It should be noted that since the results are not overwhelmingly positive, the results alone do not suggest a very high priority concern for heritable risk. However, atrazine warrants further examination due to the potential exposure to humans. Atrazine is among the most commonly occurring pesticides in ground water. It is also found in surface waters. The high frequency of atrazine detection in ground water is related to its high volume of use. More pounds of atrazine active ingredient are applied in the United States annually than any other pesticide (with the possible exception of alachlor). In addition, for applicator exposures, there are many instances where the margins of safety and margins of exposure are considered to be of toxicological concern. With this high potential for human exposure, the Agency should deal with any concern for potential health effects, heritable risks included.

Ciba-Geigy has also performed a dominant lethal test in mice (Document #005833). However, this assay was considered unacceptable despite the collection of additional information concerning this dominant lethal assay. It should be noted that

this endpoint was a concern as Adler (1980) reported a positive effect in this assay (discussed above). The Agency would like to see this result addressed. Brusick states in his review that the positive effects were found at a higher dose level than that of the submitted dominant lethal study and since there were no other confounding factors, the positive results are accepted in his analysis. Again, it should be mentioned that vehicle effects may play a role here (the positive study used olive oil, the negative study used CMC).

It is suggested for the Registration Standard that an acceptable dominant lethal assay with male mice be performed with atrazine active ingredient. Since it is the intention to reproduce the published study's results, it is highly recommended that the registrant discuss with the OPP the protocol(s) for which to perform this test (e.g. selection of doses, dose range, vehicle, animal strains). This test should be able to be completed and reported to the OPP within a one year time period.

006937

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The summary data regarding the distribution, metabolism and the elimination of atrazine were provided in this report. To this end, three separate experiments were conducted with the use of three groups of rats. Radiolabeled atrazine (triazine ring, uniformly labeled) was used by the author to measure the disposition of atrazine and/or its metabolites in the rat. The first experiment was performed to assess the distribution and elimination of atrazine in male and female rats repeatedly exposed to daily doses of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine in female rats, especially in the red blood cell. The third experiment was conducted to identify the urinary metabolites of atrazine formed by the female rat. The absorption of atrazine in male or female rats was not reported.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The rad cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to completely satisfy the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

Table 2
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Low2 (LDT2)	3.0	2	10
4 Low3 (LDT3)	7.0	2	10
5 Low4 (LDT4)	10.0	2	10
6 Mid1 (MDT1)	50.0	2	10
7 High (HDT1)	100.0	2	10

Table 3
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

B. Diet Preparation: Atrazine was was given orally to the rats (via a stomach tube) as an active ingredient or as a radiolabeled active ingredient. Animals were allowed free access to animal feed (Purina) and tap water. The animals were allowed a one-week acclimation period prior to initiation of experimentation.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Atrazine dosage regimens: Three separate experiments were conducted with the use of three groups of rats. The first experiment was performed to assess the distribution and elimination of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine, especially in the red blood cell. The third experiment was conducted to identify the atrazine metabolites formed by the rat.

1. Experiment #1. As shown in Tables 1, 2, and 3, respectively, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single oral dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single oral dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily oral doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were collected in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 2).

FIGURE 2

Digestive system		**Cardiovascular**		**Neurological**			
	Tongue		Aorta*		X	Brain*+@	
	Salivary glands*		Heart*@			Peripheral nerve*#	
	Esophagus*		Bone marrow*#			Spinal cord (3 levels)*#	
	Stomach*		Lymph nodes*		X	Pituitary*	
	Duodenum*		Spleen@			Eyes (optic n.)*#	
	Jejunum*		Thymus*				
	Ileum*	X	Red blood cell			**Glandular**	
	Cecum*	**Urogenital**				Adrenal gland*	
	Colon*	X	Kidneys*+@			Exorbital lacrimal gland#	
	Rectum*		Bladder*		X	Mammary gland*#	
X	Liver *+@		Testes*+@			Parathyroids*++	
	Gall bladder*#		Epididymides			Thyroids*++	
	Pancreas*		Prostate		**Other tissues**		
Respiratory			Seminal vesicle			Bone (femur)*#	
	Trachea*#	X	Ovaries*+@			Muscle*#@	
	Lung*@		Uterus*@			Skin*#	
	Nose^		Cervix			All gross lesions and masses*	
	Pharynx^		Fallopian tubes			Residual Carcass@	
	Larynx^					Fat@	
						Plasma (blood)@	

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution and Elimination of Atrazine and Its Metabolites

Experiment #1. The 5 male and 5 female rats were used to assess the disposition and elimination of atrazine after single or multiple oral doses of atrazine. Table 4 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the multiple dosing or the multiple exposure group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 4). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats treated with multiple oral doses of atrazine when compared to the group of rats who received a single oral dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding the percentage of ^{14}C-label that was excreted in the urine and feces (Table 4). The pattern for tissue distributed between single and multiple exposure groups were similar (Table 5) collected 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 5). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the multiple dose group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the

Table 4

Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment (taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (multiple doses)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

B. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

Another experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after multiple doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this multiple dosing experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 6). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 6. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are found: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 7). RBC concentrations appeared to be proportional (usually supralinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs were occurring. (The life span of a rat RBC is 45-56 days). The authors speculate that binding of ^{14}C-label is of a covalent nature.

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Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 8). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 6). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary and ovary. The brain had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally supralinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.

B. The Metabolism of Atrazine

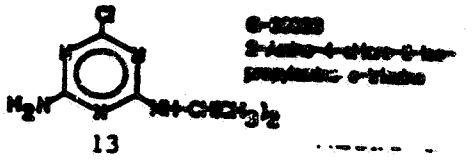
To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label.

In vivo metabolism of atrazine. The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Then the metabolites were dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Eight metabolites were identified and the major metabolites are listed below:

- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the major metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because four other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12), oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.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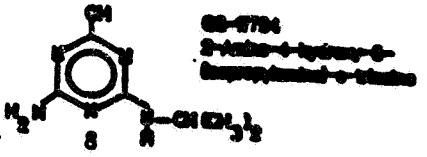
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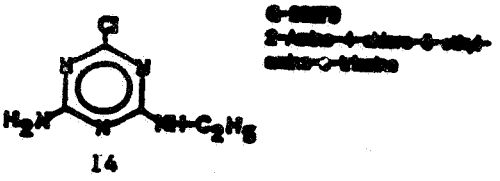
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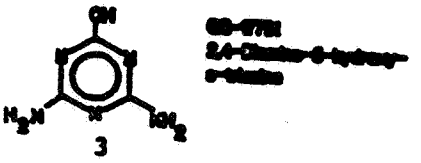
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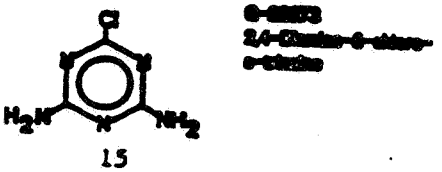
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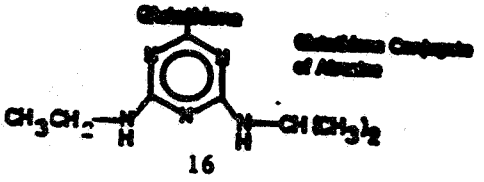
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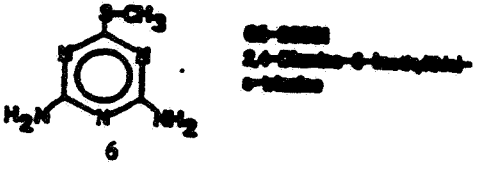
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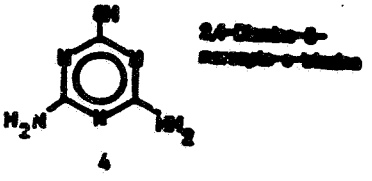
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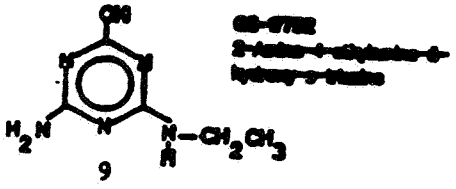
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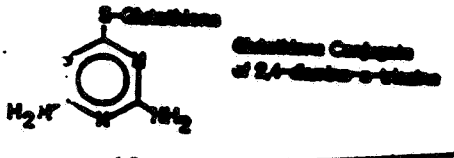
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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

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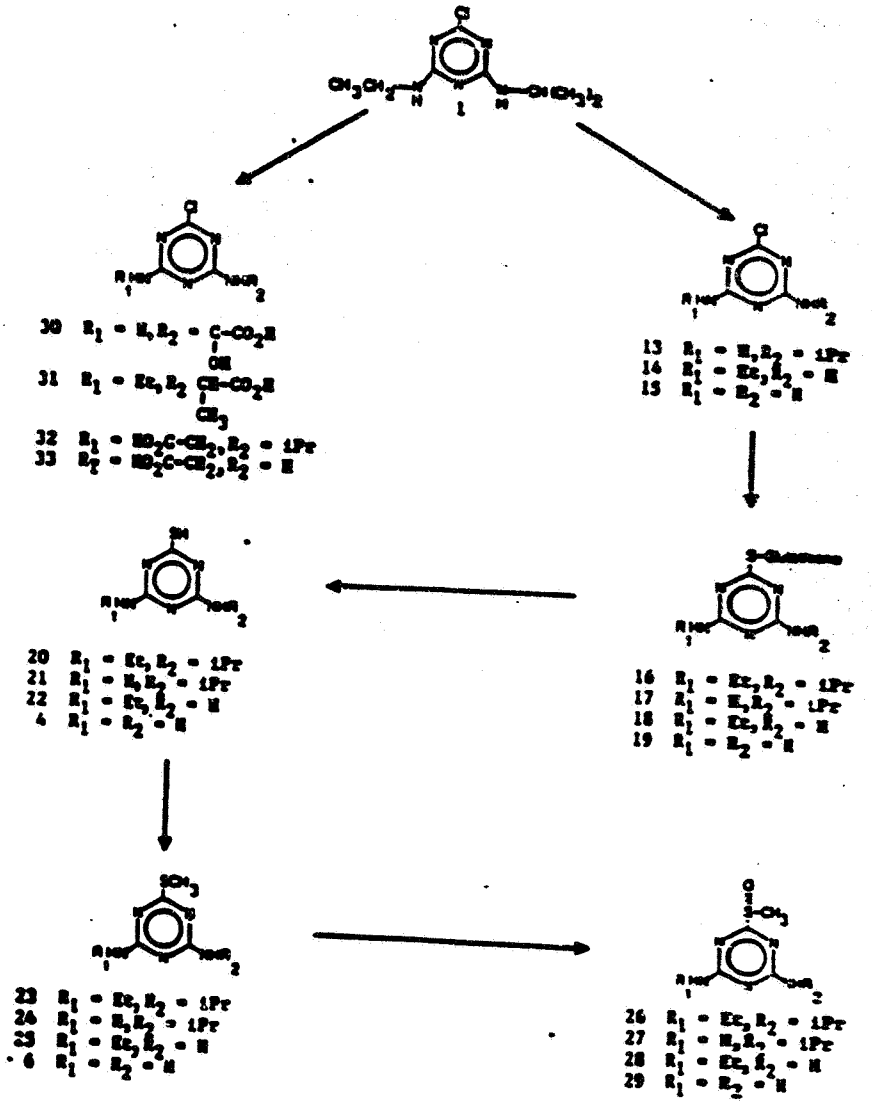

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FIGURE 2. PROPOSED METABOLIC PATHWAY FOR ATRAZINE IN THE RAT

proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

As mentioned above, the highest level of atrazine was found in the RBC. The estimated half-life of 8.14 days in RBCs (as compared to the whole body half-life of 1.61 days) indicates that extensive binding of atrazine or its metabolites in RBCs was occurring. However, after cessation of multiple exposure, the concentration of atrazine or its metabolites in RBCs declined at all doses except for the highest dose, 100 mg/kg atrazine.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

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Data Evaluation Report

006718

Compound Atrazine

Citation

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), T. Murphy, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-87098; 11/6/87, MIRD 404313-08.

This document contains the following report which describes the in life portion of the study;

Dermal absorption of ^{14}C-Atrazine in Rats, E.M. Craine, WIL Research Laboratories, Project No. WIL-82015, 11/5/87.

Reviewed by Robert P. Zendzian Ph.D. 3/24/88
Senior Pharmacologist

Core Classification Acceptable

Conclusions

Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values are 2.00, 0.53 and 0.26 % for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49 %). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours. The data indicate that absorption is approaching saturation at the high dose.

Materials

Artazine uniformly ring labeled,

low and mid doses
22.0 uCi/mg, 99.5%

high doses
2.3 uCi/mg, 99.0%

Crl:CD®BR male rats 27-41 days old from Charles River Breeding laboratories

Experimental design and methods

Dose preparation and sample analysis was performed at Ciba-Geigy and the in life portion of the study at WIL.

"The low dose was prepared by mixing throughly 4.0 mg of ^{14}C-Atrazine and 5.3 mg of the formulant (4L), then suspending the mixture in 2.0 ml of deionized water. The middose was

prepared by mixing 40 mg of ^{14}C-Atrazine and 53.0 mg of blank formulation (4L) and then suspending the mixture in 2.0 ml of deionized water."

"The 4L high dose formulation was prepared by mixing throughly 530 mg of formulant and 400.0 mg of ^{14}C-Atrazine, then suspending the mixture in 4.0 ml of water. The 80W high dose was prepared by mixing 200.0 mg of ^{14}C-Atrazine and 50.0 mg blank formulant, then suspending the mixture in 2.0 ml of deionized water.

Two groups of 16 and one group of 20 male rats were treated dermally with single doses of ^{14}C-atrazine at 0.1, 1.0 and 10.0 mg/rat (0.01, 0.1 and 1.0 mg/cm^2) respectively. Four animals at each dose were dosed with 4L formulation and exposed for 2, 4, 10 and 24 hours. The remaining four animals at 10.0 mg/rat were dosed with 80W formulation and exposed for 10 hours.

"The test material preparations were stored frozen, warmed to room temperature and sonicated 10 minutes prior to analysis and dosing on the appropriate test material application day."

The anterior dorsal hair was shaved from each rat and the area washed with acetone 24 hours prior to dosing. Test material was applied to a 2.5 x 4 cm (10cm2) area by pipette. The application site was covered with a protective device consisting of a stomahesive bandage as a wall and a filter paper cover.

Animals were individually caged in metabolism cages and total urine and feces collected.

Animals were sacrificed at the end of the exposure period. The protective device was removed and washed. The application site was washed with a detergent solution and water rinsed.

Blood, application site skin, skin under the bandage and the carcass were collected.

The following samples from each animal were sent to Ciba-Geigy for analysis;

"pipet washes, urine, feces, washes, extracts, samples from the protective coverings, gauze, blood, skin samples and carcasses,"

Results

Sample analysis for radioactivity at WIL indicated that dosing suspensions were homogenous and of the expected activity.

No compound-related effects on the rats were reported.

Dermal absorption data is summarized in Table 1 below and presented in detail in Tables III - VI of the report.

Table 1. Summary of dermal absorption data. All values are means of 4 animals. All animals dosed with 4L formulation except as noted. Data from Tables III - VI of the report.

Dose (mg/cm^2)	Exposure (hours)	Absorbed[a] (%)	(%/hr)	(mgx10-5)	On skin[b] (%)	Unabsorbed[c] (%)
0.01*	2	0.68	0.34	6	23.53	77.25
0.009†	4	1.24	0.31	11	20.56	71.88
	10	2.00	0.20	18	24.87	69.51
	24	4.93	0.21	44	20.72	69.02
0.1	2	0.21	0.11	20	25.06	71.55
0.095	4	0.36	0.09	34	18.97	75.72
	10	0.53	0.05	50	21.10	78.93
	24	1.26	0.05	119	29.04	67.43
1.0	2	0.13	0.06	107	11.24	88.67
0.82	4	0.09	0.02	74	14.69	88.00
	10	0.26	0.03	213	10.49	89.29
	24	0.21	0.01	172	9.58	91.03
1.0 80W	10	0.24	0.02	244	8.81	89.15
1.02						

* Nominal dose.
† Applied dose.
a. Total of blood, carcass, urine and feces.
b. Total of skin I and skin II.
c. Total of bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, gauze A, gauze B and cage wash.

Discussion

The percent of dose absorbed followed the most common pattern of absorption with the percent increasing with time and decreasing with increasing dose. Significant quantities of test material remained on/in the skin following soap and water wash. There are clear indications that the process is approaching saturation at the high dose in that;

1. The percent absorbed per hour decreased with time in each dose and the proportionate decrease was larger with increasing dose.

2. As the dose increased the total quantities absorbed increased proportionately less per dose increase.

3. The quantity on/in the skin increased ten fold from 0.01 to 0.1 mg/cm^2 but only five fold from 0.1 to 1.0 mg/cm^2.

For regulatory purposes the test material which remains on/in the skin after soap and water wash is considered absorbable. For risk assesments the percent absorbed is added to the percent on/in the skin to determining quantity absorbed. However, the possibility exists that the relatively large quantity remaining on/in the skin is an artifact of the experimental procedure. A recent study, designed to determine if the material remaining on/in the skin after washing could be absorbed, showed that 2 to 3 times more material could be washed from the skin of living animals then from the skin of recently sacrificed animals. In this study the animals were sacrificied before washing the application site.

This possibility may be tested by treating 4 animals per dose for 10 hours exactly as was done in this study but washing the appliction site before sacrificing the animals. The ten hour exposure time is suggested as modeling a worker who washes at the end of the working day.

ATRAZINE 080803

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C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine (Wake Forest University). The evaluation was limited because of the low number of rats used in each group. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from (1) SRI International, the subcontracting laboratory where the distribution of radiolabeled atrazine was studied and (2) Agrisearch Incorporated, another subcontracting laboratory where the amount of radiolabeled atrazine was measured.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study. Rat # 5065 (given 3 mg/kg atrazine for 3 days) favored its right side, and upon examination, the lungs were found to be "present in the lower thoracic area."

B. Experimental Protocol: This experiment was performed to assess in further detail the dose-dependent distribution of atrazine, especially in the red blood cell. As listed in Table 1, in an effort to study in more detail the toxicokinetic disposition of ^{14}C-atrazine as a function of the dose of atrazine and the time of sacrifice, six groups of female Sprague-Dawley rats were treated with ^{14}C-atrazine while another group of female rats served as a control group. The groups of rats were dosed daily for 10 consecutive days at 0 mg/kg (vehicle only), 1 mg/kg, 3 mg/kg, 7 mg/kg, 10 mg/kg, 50 mg/kg, and 100 mg/kg ^{14}C-atrazine. The vehicle was an aqueous solution of corn starch/polysorbate-80.

Urine and feces were collected daily. At 24, 48, 72, 96, 144, 192, 219, 240, 264 and 288 hours, blood samples were obtained via orbital puncture. Five milliliters of blood were collected by aortal puncture at sacrifice. The tissues selected for determining the distribution of ^{14}C-label at each dose are listed in Figure 1. One of the two animals in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 1).

FIGURE 1

	Digestive system		Cardiovascular		Neurological
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*		Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
	Ileum*	X	Red blood cell		Glandular
	Cecum*		Urogenital		Adrenal gland*
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*	X	Mammary gland*#
X	Liver *+@		Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate		Other tissues
	Respiratory		Seminal vesicle		Bone (femur)*#
	Trachea*#	X	Ovaries*+@		Muscle*#@
	Lung*@		Uterus*@		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes		Residual Carcass@
	Larynx^				Fat@
					Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

The experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after exposure for 10 days to a number of doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 2). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 2. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are reported: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

Table 2

Plasma Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table VIII)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
4	0.068	0.061	0.063	0.375	0.741	0.562	1.062	1.164	9.291	8.279	27.104	22.298
8	0.186	0.181	0.452	0.615	1.058	1.884	2.009	1.593	7.161	.1297	28.946	23.101
2	0.469	0.405	1.383	1.468	3.267	3.248	4.168	3.957	20.911	17.778	40.320	57.877
6	0.506	0.596	1.808	1.845	3.311	3.747	4.165	4.661	21.249	24.448	52.271	56.580
44	0.582	0.594	2.150	2.608	4.225	3.359	5.069	5.109	25.169	24.604	59.751	69.514
92	0.560	0.658	1.668	1.941	4.066	3.533	5.343	4.725	23.437	27.682	48.671	44.420
19	0.583	--	--	1.406	3.748	--	5.067	--	21.351	--	51.715	--
40	--	0.185	0.703	--	--	1.628	--	3.099	--	26.413	--	29.566
64	--	0.144	0.789	--	--	1.371	--	1.713	--	13.352	--	17.682
88	--	0.117	0.340	--	--	0.868	--	1.600	--	8.302	--	14.775

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 3). RBC concentrations appeared to be proportional (usually superlinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs was occurring. (The life span of a rat RBC is 45-56 days). The author speculates that binding of ^{14}C-label is of a covalent nature.

Table 3

Red Blood Cell Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(Taken from Table IX)

Dose	1 mg/kg		3 mg/kg			7 mg/kg			10 mg/kg			50 mg/kg			100 mg/kg		
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073					
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72					
Time (hrs.)																	
24	0.93	0.54	2.57	4.67	5.19	6.39	7.31	7.81	50.87	36.59	109.06	70.38					
48	1.48	1.27	4.29	8.48	12.82	14.17	20.72	15.88	124.35	87.00	234.05	190.48					
72	2.64	2.77	--	--	19.98	23.08	31.55	26.71	177.56	129.41	292.78	275.59					
96	3.51	2.76	21.47	22.01	26.65	30.38	39.07	37.59	225.49	160.46	474.56	305.37					
144	5.18	4.24	--	--	37.07	30.78	60.65	53.79	358.75	289.12	881.41	649.45					
192	6.61	5.04	23.44	11.63	43.78	50.05	63.84	63.85	415.80	362.30	695.14	529.04					
219	10.03	--	--	21.20	54.98	--	83.92	--	307.74	--	517.23	--					
240	--	5.98	18.57	--	--	51.45	--	85.63	--	324.18	--	551.40					
264	--	5.41	18.11	--	--	46.65	--	67.83	--	318.95	--	605.28					
288	--	5.34	18.50	--	--	50.77	--	41.26	--	271.48	--	611.42					

Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 4). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 2). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary, ovary and brain. The pectoral and inguinal mammary glands had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally superlinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.